

REMARKS

Applicant thanks the Examiner for her consideration of applicant's September 8, 2009 Response and her withdrawal of the previous rejections and objections.

Claim Amendments

Applicant, as discussed in detail below, has amended independent claims 45, 60 and 65 (and the claims that depend therefrom) to clarify that the recited environmental parameter does not include bacteria. This amendment is supported in the specification, for example, at page 13, line 23 - page 14, line 18.

Applicant has restored claims 51-56 and designated them as withdrawn, not canceled. These claims were inadvertently canceled in the December 23, 2008 response to the July 14, 2008 Office Action. Claims 51-56 are not readable upon the elected species. However, applicant requests that, upon allowance of the generic claims from which claims 51-56 depend, the Examiner consider claims 51-56 and allow them, as provided by 37 C.F.R. § 1.141.

Applicant has amended the dependencies of withdrawn claims 51-56 to reflect the claim amendments made over the course of prosecution since their inadvertent cancellation. Applicant has also amended withdrawn claims 51-56 to recite “. . . wherein the environmental parameter is. . .” to fix antecedent basis. Finally, applicant has amended withdrawn claim 51 to correct a grammatical error.

With the amendments, claims 45, 47-56, and 58-69 are pending (claims 51-56 and 69 being withdrawn).

Claim Interpretation

The Examiner asserts that “environmental sample” is to be interpreted as any sample ; “environmental parameter” is to be interpreted as any parameter ; “abundance of gene segments” is to be interpreted as either the presence/absence of gene segments or a number of gene sequences ; and “parameter of interest is surface oil or natural gas deposit” is to be interpreted as any parameter pertaining to oil or gas. Applicant traverses in part.

(i) environmental parameter

The Examiner interprets “environmental parameter” to mean any parameter, including bacteria defined by a 16S rRNA tag. Applicant disagrees. In the claimed invention, the abundance of the 16S rRNA is used as a proxy for the abundance of the bacteria defined by said gene segment and to determine correlations with the abundance of an environmental parameter. A person skilled in the art would not interpret “environmental parameter” to mean the bacteria defined by the 16S rRNA tag. Otherwise the bacteria defined by the 16S rRNA tag and the environmental parameter that correlates to the 16SrRNA tag would be the same thing! No correlation would be possible.

Indeed, the specification itself states:

Microbes inhabit virtually all niches including extreme environments . . . In order to prevail under such diverse conditions, microbes have made remarkable adaptations and have attained the ability to utilize unusual carbon and mineral resources that are immediately available. These physiological and metabolic adaptations that enable some microbes to inhabit a particular niche may also restrict their distribution to such areas. Numerous examples of environmental parameters that lead to restrictions of microbial distribution are well known and are usually dictated by a species' specific metabolic program (e.g. obligate nature of the carbon, nitrogen and energy source).

Microbes that have highly defined nutrient requirements are likely to have a restricted distribution in the environment. Thus, the microbes' dependence on the presence of a particular resource to proliferate can serve as the basis for an

assay to identify the presence, and characterize the distribution, of various features in the environment, such as biological, chemical and geochemical features. In other words, microbes can function as environmental biosensors.

In one aspect of this invention, the ability of microbes to function as environmental biosensors is used to identify particular environmental states. In a preferred embodiment, a profile of a microbial population is used to identify one or more parameters of a particular environmental state. (emphasis added)
(Specification, page 13, line 23 - page 14, line 18)

Thus, the specification makes clear that the environmental parameter and the bacteria defined by the 16S rRNA gene segment are distinct and separate from each other. Therefore, a skilled worker, reading the claims in light of the specification, would not interpret “environmental parameter” to mean the bacterial population defined by the 16S rRNA gene segment.

Moreover, in the context of the claims as a whole, as well as the specification, the abundance of a bacterial population, quantitated by the abundance of said 16S rRNA gene segment that defines said bacteria, is what is correlated with the abundance of an environmental parameter (*see* step (f) of claims 45 and 60). That is, the abundance of the 16S rRNA gene segment is used as a *proxy* for the abundance of the bacteria defined by said gene segment, which, once determined, is correlated with the abundance of the *non-bacterial* environmental parameter. If, as the Examiner alleges, the abundance of the bacterial species defined by said 16S rRNA gene segment was considered to be an environmental parameter, the correlation required in the invention would essentially be comparing one measure to itself. As such, for this reason also, and despite the Examiner's assertions to the contrary, a person skilled in the art would not interpret “environmental parameter” to mean the bacteria defined by the 16S rRNA gene segment.

Notwithstanding the above, applicant has amended independent claims 45, 60 and 65 to clarify the term “environmental parameter” as not including bacteria. This is not a

narrowing amendment. It clarifies the original scope and meaning of the claims when read by the skilled worker in the context of the claims as a whole and the specification.

(ii) abundance of gene segments

The Examiner interprets “abundance of gene segments” to mean the presence/absence of gene segments. Applicant disagrees. The term “abundance” reflects a quantitative measure of the amount of an entity. By contrast, the terms “presence” or “absence” reflect a qualitative expression of yes or no. Thus, the terms have very different meanings and the skilled worker would not construe one to include the other, as the Examiner has done. See, e.g., Figures 5, 6, 7, 14-17 which show that abundance of the 16S rRNA gene segments, as determined for the claimed invention, is a range of values rather than merely the absolutes of absence or presence. The claims themselves make this definition of abundance clear. They recite “determining” the abundance of the gene segment. That is a quantitative measure. If abundance meant qualitative presence/absence (which it does not), the proper terminology would have been “detecting” the gene segment. *See infra* at pages 19-20, for applicant's discussion of Leu's “detection” of the presence of sulfate reducing bacteria. Should the Examiner believe that further clarification is necessary or that a phrase other than “determining the abundance” would be preferred, applicant stands ready to consider such suggestion and to amend the claims as appropriate.

(iii) “parameter of interest is surface oil or natural gas deposit”

As a preliminary matter, none of the pending claims recites “parameter of interest is surface oil or natural gas deposit.” Rather, claims 50, 64 and 66 recite “the environmental parameter is subsurface oil or natural gas.” That aside, Examiner interprets this phrase as including any parameter pertaining to oil or gas, including bacteria defined by the 16S rRNA tag.

Applicant disagrees for the reasons discussed above regarding the Examiner's interpretation of "environmental parameter." As also discussed above, applicant's clarifying amendment of independent claims 45, 60 and 65 makes clear that the Examiner's interpretation of this phrase is mistaken.

Rejections

35 U.S.C. § 102 – Anticipation

Claims 45, 47, 58-61, 65 and 67-69 stand rejected under 35 U.S.C. §102(a) as being allegedly anticipated by Matsuki et al. (1999) Appl. Environ. Microbiol., 65: 4506-4512 ("Matsuki"). The Examiner contends that Matsuki refers to a culture-independent method of determining the abundance of an environmental parameter in an environmental sample, and argues that each step of independent claims 45, 60 and 65, as the Examiner has interpreted them, is recited in Matsuki. In particular, the Examiner applies Matsuki to the steps of applicant's independent claims as follows:

Step (a) of claims 45 and 60, "providing a first plurality of environmental samples at least some of which samples contain the environmental parameter," is allegedly anticipated by p. 4506, last paragraph and Table 1 of Matsuki;

Step (b) of claims 45 and 60, "isolating a plurality of genomic DNAs from each of the samples provided in step a," is allegedly anticipated by p. 4508, fifth paragraph of Matsuki;

Step (c) of claims 45 and 60, "isolating a plurality of 16S rRNA gene segments from each plurality of genomic DNAs isolated in step b," is allegedly anticipated by p. 4507, first paragraph and p. 4508, fifth paragraph of Matsuki;

Step (d) of claims 45 and 60, “determining the abundance of each of said 16S rRNA gene segments. . .,” is allegedly anticipated by p. 4507, first and second paragraphs; Table 3; and Figure 1 of Matsuki;

Step (e) of claims 45, and 60, “determining the abundance of the environmental parameter. . .,” is allegedly anticipated by Table 3 and Figure 1 of Matsuki;

Step (f) of claims 45, and 60, “correlating the abundance of each 16S rRNA gene segment. . .with the abundance of the environmental parameter. . .,” is allegedly anticipated by Table 3, Figure 1, and pages 4508-4509 (last and first paragraphs, respectively) of Matsuki;

Step (g) of claims 45 and 60, “selecting at least one 16S rRNA gene segment whose abundance correlates to the abundance of said environmental parameter, as determined in step f,” is allegedly anticipated by page 4508, fifth paragraph of Matsuki;

Step (h) of claim 45 (also step (a) of claim 65), “providing an environmental sample set of at least one environmental sample,” is allegedly anticipated by page 4507, last paragraph and page 4508, first paragraph of Matsuki;

Step (i) of claim 45 (also step (b) of claim 65), “isolating a plurality of genomic DNAs from each environmental sample of the environmental sample set provided in step h,” is allegedly anticipated by page 4508, second paragraph of Matsuki;

Step (j) of claim 45 (also step (c) of claim 65), “determining the abundance of said 16S rRNA gene segment selected in step g in each plurality of genomic DNAs isolated in step i,” is allegedly anticipated by page 4508, second paragraph, page 4510, second paragraph, and page 4511, first paragraph of Matsuki; and

Step (k) of claim 45 (also step (d) of claim 65), “inferring the abundance of the environmental parameter in each environmental sample of the environmental sample set provided in step h based upon the abundance of said 16S rRNA gene determined in step j in each environmental sample of the environmental sample set provided in step h,” is allegedly anticipated by page 4511, third paragraph of Matsuki.

The Examiner further contends that claim 60 is anticipated by Matsuki, which allegedly recites designating the fragments amplified by PCR primers as indicators of the presence of bacteria (page 4508, fifth and sixth paragraphs, page 4509, first paragraph, Figure 1).

The Examiner further contends that some of the claims dependent from claim 45, 60 or 65 are anticipated by Matsuki. According to the Examiner, Matsuki recites that there was a 100% correlation between the presence of the PCR amplicon for the Bifidobacterium strain 16S rRNA and the presence of said bacteria stain in the sample. The Examiner, thus, contends that claims 47 and 61 are anticipated by Matsuki. The Examiner further contends that because Matsuki recites PCR (*see* page 4507, (second paragraph) and 4508 (second paragraph)), and because PCR relies on hybridization of primers to target sequences, Matsuki anticipates claims 58, 59, 67 and 69. Finally, the Examiner contends that Matsuki recites cell cultures and a physiological condition (*see* page 4506, second paragraph), and that, thus, Matsuki anticipates claim 69.

Applicant traverses the Examiner's novelty rejection of the independent and dependant claims for at least the reasons detailed below.¹

Applicant respectfully disagrees with the Examiner's contention that step (d) of claims 45 and 60 (also step (j) of claim 45 and step (c) of claim 65) "determining the abundance of each of said 16S rRNA gene segments. . ." is anticipated by Matsuki. Matsuki at best determines the absence/presence of the gene segment. That does not constitute "determining the abundance" of the gene segment. *See supra* at page 10, last paragraph -- page 11, first paragraph. For example, Figures 5, 6, 7, and 14-17 of the instant application show that abundance of the 16S rRNA gene segments is measured as a range of values, not absence vs. presence. For this reason alone, Matsuki does not anticipate applicant's claims. Applicant requests, therefore, that the Examiner reconsider and withdraw this rejection.

Applicant also respectfully disagrees with the Examiner's contention that step (e) of claims 45 and 60, "determining the abundance of the environmental parameter. . ." is anticipated by Table 3 and Figure 1 of Matsuki. First, as applicant has demonstrated above, Matsuki does not determine the abundance of anything. At best, Matsuki is a yes/no, presence/absence analysis of bacteria. Second and most importantly, the Examiner mistakenly considers the bacterial species defined by the 16S rRNA tags as an "environmental parameter" of the claims. As applicant has explained above (*see supra* at pages 9-10), the abundance of the 16S rRNA tags are used in the methods of the invention as a proxy for the abundance of bacteria.

¹ The fact that applicant has relied on only these two reasons to overcome the rejection is not an admission or suggestion that other reasons do not also exist.

The bacteria is not the environmental parameter itself. Applicant has amended the claims to clarify that distinction.

Because the “environmental parameter” of the claimed invention cannot be interpreted as the bacterial population defined by the 16S rRNA gene segment (and applicant’s claim amendments clarify that fact), even if abundance meant presence/absence, which it does not, Matsuki does not refer to, or suggest, correlating the 16S rRNA gene segments with the environmental parameter (i.e., step (f) of claims 45 and 60), or any of the subsequent steps (i.e., steps (g)-(k) of claim 45, steps (g)-(h) of claim 60, as well as steps (a)-(d) of claim 65).

For at least the reasons discussed above, independent claims 45, 60 and 65 are not anticipated by Matsuki. Therefore, claims 47, 58, 59, 61, and 67-69, which depend from claims 45, 60 or 65, are also not anticipated by Matsuki.

Applicant requests that the Examiner reconsider and withdraw the anticipation rejection.

35 U.S.C. § 103 – Obviousness

(i) *Matsuki and Clarke*

Claims 48, 49, 62 and 63 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Matsuki and Clarke et al., J. Nutr., (1990) 120:218-224 (“Clarke”). The Examiner acknowledges that Matsuki does not teach calculating coefficients to analyze data. The Examiner, however, contends that claims 48, 49, 62 and 63 are, nevertheless, obvious because it was well known in the art at the time of applicant’s invention how to determine a correlation coefficient between two variables, e.g., a level of gene expression and level of sugar, as shown by Clarke (page 222, Figure 4). Applicant traverses.

As discussed above, claims 45 and 60, from which claims 48, 49, 62 and 63 depend, differ from Matsuki in a number of important and patentable ways beyond the representation of data in the form of correlation coefficients. Therefore, even with the knowledge of determining correlation coefficients, as allegedly shown by Clarke, claims 48, 49, 62 and 63 would not have been obvious to the skilled worker. Clarke in no way remedies the deficiencies in Matsuki in the context of the amended claims.

(ii) Leu and Devereux

Claims 45, 50, 60 and 64-66 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Leu et al. (1998) *Anaerobe*, 4:165-174 ("Leu") and Devereux et al. (1994) *Appl. Environ. Microbiol.* 60: 3437-3439 ("Devereux").

In particular, the Examiner in the context of Leu contends that:

Step (a) of claims 45 and 60, "providing a first plurality of environmental samples at least some of which samples contain the environmental parameter," is allegedly anticipated by p. 166, paragraph 4 and Table 1 of Leu, where the samples were obtained from different oil fields ;

Step (b) of claims 45 and 60, "isolating a plurality of genomic DNAs from each of the samples provided in step a," is allegedly anticipated by p. 167, paragraph 2 of Leu;

Step (c) of claims 45 and 60, "isolating a plurality of 16S rRNA gene segments from each plurality of genomic DNAs isolated in step b," is allegedly anticipated by p. 167, paragraphs 3-5 of Leu ;

Step (d) of claims 45 and 60, “determining the abundance of each of said 16S rRNA gene segments. . .,” is allegedly anticipated by p. 168, paragraphs 4-7 and p. 169, paragraphs 1-3 of Leu;

Step (e) of claims 45, and 60, “determining the abundance of the environmental parameter. . .,” is allegedly anticipated by p. 171, paragraph 5 of Leu, where the presence of *Desulfotomaculum* genus was detected in original samples; and

Step (f) of claims 45, and 60, “correlating the abundance of each 16S rRNA gene segment. . .with the abundance of the environmental parameter. . .,” is allegedly anticipated by p. 171, paragraph 5 and p. 172, paragraph 1 of Leu.

Further, the Examiner contends that while Leu does not specifically teach the steps (g)-(k) of claim 45, it allegedly suggests performing such steps, i.e., analysis of 16S rRNA sequences of bacterial populations obtained from oil fields, determining the most common and populous bacterial species, and using probes based on their rRNA sequences to analyze samples from different oil fields. The Examiner then looks to Devereux to allegedly find the expectation of success for this supposed approach. In that context, the Examiner contends that Devereux determined sequence diversity of bacteria from marine sediments without enrichment protocols and determined 16S rRNA sequences to be used for the detection of the different species. Based on the above, the Examiner contends that it would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to have used the method of Leu and Devereux to obtain the abundance of bacteria in oil fields. Applicant traverses.

Leu at best determines the absence/presence of the gene segment. As explained above, that is not “determining the abundance.” Also as explained above, bacteria defined by the 16S rRNA gene segment is not an “environmental parameter”. For these reasons alone, Leu

does not anticipate or suggest steps (d)-(f) of claims 45 and 60. In addition, and for same reasons, Leu does not suggest performing steps (g)-(k) of claim 45 (which also correspond to step (g) of claim 60 and claims (a)-(d) of claim 65). Devereux, which merely identifies some phylogenetic groups of bacteria with their respective 16S rRNA sequences, fails to remedy these deficiencies of Leu. For at least these reasons, claims 45, 60 and 65 (and claims 64 and 65 that depend therefrom) are not obvious over Leu and Devereux.

The Examiner raised a similar obviousness rejection based on Leu in the October 18, 2007 Office Action (steps (g)-(k) of claim 45, as currently pending, are broadly similar to steps (a)-(d) of claim 45, as examined in the October 18, 2007 Office Action). Applicant understood this rejection to have been successfully overcome by applicant's April 18, 2008 response. Indeed, the Examiner stated in the July 14, 2008 Office Action that applicant's amendments overcame all previous rejections. See page 2 of the July 14, 2008 Office Action.

The following is an excerpt from applicant's April 18, 2008 response regarding the Examiner's contentions that the claimed invention was obvious over Leu.

The methods recited in Leu identify thermophilic SRBs (sulfate reducing bacteria) in samples taken from oil reservoirs. They do not identify reservoirs with hydrogen sulfide. In fact, according to Leu, SRB-related sequences are present in all oil field samples (e.g., see Introduction, p. 166, third paragraph). Leu also recites that SRBs appear to be common and widely distributed over oil field environments, presumably even in environments that lack hydrogen sulfide (see page 170, fourth paragraph). Therefore, according to Leu, the presence of the SRBs is completely uncorrelated with the presence of hydrogen sulfide. As such, Leu does not suggest or allow making inferences regarding the abundance of hydrogen sulfide in oil fields based on the presence of SRBs.

Finally, Leu recites detecting, but not determining the abundance of, a nucleic acid marker sequence within the genomic DNA collected from the sample. In contrast, the claimed invention involves correlating the abundance of a nucleic acid marker sequence with the abundance of an environmental parameter (see, e.g., page 24, line 12, page 27, lines 12-14, and Figs. 5, 6, 7 in the specification).

For the above reasons, applicant requests that the Examiner reconsider and withdraw the 103(a) rejection.

Applicant believes that these arguments remain relevant.

The above excerpt from the April 18, 2008 response above also highlights the importance of the term “abundance” in the context of the claimed invention as meaning something far more than presence/absence. Many bacteria survive at low levels even under conditions in which they would not flourish. As such, a bacterial species can be present, at least at low levels, over a wide range of different environmental conditions. Consequently, merely the detection of a particular 16S rRNA gene segment (a yes/no or presence/absence answer) would provide no correlative information regarding environmental parameters. This point is illustrated in Leu. As explained applicant's the April 18, 2008 response, Leu recites that SRBs appear to be common and widely distributed over oil field environments, presumably even in environments that lack hydrogen sulfide (*see* page 170, fourth paragraph). This means that the presence of the SRBs is not correlated with the presence of hydrogen sulfide.

For all of these reasons, claims 45, 60 and 65 (and claims 50, 64 and 66 that depend therefrom) are not obvious over Leu and Devereux.

Applicant requests that the Examiner reconsider and withdraw the obviousness rejections.

CONCLUSION

Applicant requests consideration of the amended claims in view of the foregoing remarks and that the Examiner allows amended claims 45, 47-56, and 58-69.

Should the Examiner feel that a telephone conference with applicant's representative would be helpful, she is invited to telephone the undersigned at any time.

Respectfully submitted,

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